PB86-191392

Methods for Isolating 'Campylobacter jejuni' from Low-Turbidity Water

(U.S.) Veterans Administration Medical Center
Denver, CO

Prepared for

Environmental Protection Agency, Cincinnati, OH

1986

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/J-86/027	2.
3. R PB86-191392	
4. TITLE AND SUBTITLE METHODS FOR ISOLATING CAMPYLOBACTER JEJUNI FROM LOW-TURBIDITY WATER (Journal Version)	5. REPORT DATE 1986
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) M.J. Blaser[1] and H.J. Cody[2]	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS [1]Veterans Adminis. Med. Center, Denver, CO, 80220 [2]Univ. Colorado School of Medicine, Denver, CO 80262	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO. CR 810169 byproduct
12. SPONSORING AGENCY NAME AND ADDRESS Water Engineering Research Laboratory-Cincinnati, OH Office of Research and Development U.S. Environmental Protection Agency Cincinnati, OH 45268	13. TYPE OF REPORT AND PERIOD COVERED Journal Article
	14. SPONSORING AGENCY CODE EPA/600/14

15. SUPPLEMENTARY NOTES
Project Officer, J. Hoff, 513-569-7331
Applied and Environmental Microbiology, 51(2):312-315, Feb. 1986

16. ABSTRACT

Membrane filtration methods were developed and evaluated for the quantitative recovery of Campylobacter jejuni from environmental waters of low turbidity. The best of the studied procedures involved passaging the test water through a filter (pore size, 0.45 um) and plating it facedown on Campylobacter-selective agar. The filter was removed after overnight incubation, and the plate was streaked for isolation and then reincubated. This technique could be useful in the routine monitoring of finished waters for C. jejuni or during investigations of suspected waterborne outbreaks for water of low turbidity.

KEY WORDS AND DOCUMENT ANALYSIS

17. a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 6
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA/600/J-86/027
JOURNAL ARTICLE

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1986, p. 312–315
0099-2240/86/020312-04$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 51, No. 2

Methods for Isolating *Campylobacter jejuni* from Low-Turbidity Water

MARTIN J. BLASER[1,2]* AND HENRY J. CODY[1]

Infectious Disease Section, Veterans Administration Medical Center, Denver, Colorado 80220,[1] and Division of Infectious Diseases, Department of Medicine, University of Colorado School of Medicine, Denver, Colorado 80262[2]

Received 26 July 1985/Accepted 20 November 1985

Membrane filtration methods were developed and evaluated for the quantitative recovery of *Campylobacter jejuni* from environmental waters of low turbidity. The best procedure studied involved passaging the test water through a filter (pore size, 0.45 μm) and plating it facedown on *Campylobacter*-selective agar. The filter was removed after overnight incubation, and the plate was streaked for isolation and then reincubated. This method, with or without prefiltration through 5.0- and 0.6-μm-pore-size membranes consistently resulted in the recovery of 30 *C. jejuni* CFU/250 ml of seeded natural waters. The other methods, plating the final filter face-up or preincubation of the filter in an enrichment medium, were not as sensitive. The technique described above could be useful in the routine monitoring of finished waters for *C. jejuni* or during investigations of suspected waterborne outbreaks for water of low turbidity.

Campylobacter jejuni and closely related thermophilic organisms are important causes of acute diarrheal disease in the United States and throughout the world (5, 20). The ultimate reservoir for these organisms is the intestinal flora of a wide variety of wild and domesticated mammals and fowl (6). The contamination of surface waters with their feces may result in the presence of *Campylobacter* spp., and contamination may persist for prolonged periods (4).

Contaminated water has been shown to be a vehicle for the transmission to humans of campylobacter infection, either in large outbreaks or in sporadic cases (6, 15, 21). In virtually every case, infection occurred after the consumption of untreated or improperly treated surface waters of low turbidity (6). However, in only a small proportion of outbreaks have campylobacter organisms been isolated from the implicated water source. In part, this may be because sampling often was done weeks after the outbreak peaked. It also may be because of the difficulties in isolating the organisms from water (10, 14, 18). Our goal for this investigation was to compare several methods for efficiency in recovering *C. jejuni* from water. Since virtually all human cases have occurred after ingestion of finished water or water of low turbidity, our efforts focused on methods of isolating *C. jejuni* from such sources.

MATERIALS AND METHODS

***C. jejuni* seed preparation.** Stool specimens from a mouse chronically infected with *C. jejuni* PEN1 (3) were mixed using a Vortex mixer (The Vortex Manufacturing Co., Cleveland, Ohio) with 2 ml of brucella broth (BBL Microbiology Systems, Cockeysville, Md.) (19). A sample was streaked for isolation on a campylobacter blood agar plate (2) (CBAP; BBL Microbiology Systems) and incubated at 42°C for 24 h in a zip-lock plastic bag containing 85% nitrogen, 10% carbon dioxide, and 5% oxygen. After incubation, *C. jejuni* colonies were subcultured onto CBAP, and a PEN1 colony was isolated. The isolated colony was grown in a biphasic medium at 42°C for 24 h (19). The resulting growth was pelleted by centrifugation from the liquid phase, and the cell pellet was washed three times in 5 ml of sterile distilled water. This procedure consistently provided a concentration of 5×10^8 to 5×10^9 *C. jejuni* CFU/ml in the final seed. All determinations were done in triplicate.

Seeding of water samples. The seed culture was diluted 10-fold in sterile distilled water to yield concentrations ranging from 10^7 to 10^0 CFU/ml (*C. jejuni* remains viable for days without a reduction in counts in water [4]). Of each dilution used, 1 ml was added to identical 999- or 1,499-ml samples of sterile distilled water or natural source water and magnetically stirred to distribute the cells. To determine the original concentrations, 3 0.1-ml samples from each dilution were spread-plated on CBAP.

Water samples. Distilled water (type I reagent grade water [Millipore Laboratory Water Purification Systems; Millipore Corp., Bedford, Mass.]), free from trace impurities at the limits of detection (18 MΩ-cm resistance at 25°C), was autoclave-sterilized. Natural source water for most studies was obtained from a Colorado stream (Bear Creek; elevation, 8,000 ft [1 ft = 30.48 cm]) of relatively low turbidity. A clean 20-liter carboy (Nalgene Labware Div., Nalge/Sybron Corp., Rochester, N.Y.) was completely submerged in the creek, and the sample water was returned on ice to the laboratory, where the following tests were performed by standard methods within 4 h (1): standard plate count, total coliforms, fecal coliforms, and turbidity. For preliminary studies, water was obtained from a high mountain stream (elevation, 11,000 ft) and assessed as described above. Stream waters were cultured for the presence of *C. jejuni* before the seeding trials were done, and samples were stored in the dark at 4°C until they were used.

Seed recovery. Seeded distilled water samples were passed into a sterile suction flask through filters with a pore size of 0.6 μm (Nuclepore Corp., Pleasanton, Calif.) supported by a sterile suction flask apparatus (Millipore Corp.,). The filtrate then was passed through a 47-mm-diameter filter (pore size, 0.45 μm, Millipore Corp.). The filtrate and the 0.6-μm filter were discarded, and the 0.45-μm filter was aseptically cut in thirds. One third of the filter was placed facedown on CBAP, one third was placed faceup, and one third was placed into 5 ml of Oosterom medium (16). The medium was modified as to contain fluid thioglycolate (Difco Laboratories, Detroit, Mich.) with 5% sheep blood and 1.5% ox bile (Bacto-Oxgall;

* Corresponding author.

TABLE 1. Recovery of *C. jejuni* from seeded sterile water, using several filtration and enrichment methods

Mean no. of organisms per filter segment	Recovery[a] of *C. jejuni* with filter:					
	0.45 μm pore			0.6 μm + 0.45 μm pore		
	Face up	Face down	Into enrichment broth	Face up	Face down	Into enrichment broth
2.3×10^6	2/2[a]	2/2	2/2	1/1	1/1	1/1
2.0×10^5	5/5	5/5	5/5	2/2	2/2	2/2
2.0×10^4	5/5	5/5	5/5	2/2	2/2	2/2
1.8×10^3	6/6	6/6	6/6	2/3	3/3	3/3
1.8×10^2	5/6	6/6	5/6	0/3	2/3	2/3
1.8×10^1	4/6	6/6	1/6	0/3	1/3	2/3
1.6×10^0	3/4	2/4	0/4	0/2	1/2	0/2

[a] Denominator refers to the number of trials at that dilution; numerator refers to number of trials when *C. jejuni* was isolated.

Difco), and the following antimicrobial agents per milliliter: vancomycin (40 μg), polymyxin B (10 IU), cephalothin (15 μg), and amphotericin B (2 μg). A second seeded water sample was passed only through the 0.45-μm filter, and thirds of this filter were handled as described above. For each aliquot of seeded natural water, a sample was passed sequentially through a 5.0-μm-pore, 47-mm-diameter filter, a 0.6-μm-pore, 47-mm-diameter filter, and a 0.45-μm-pore filter. The filtrate and the 5.0- and 0.6-μm-pore filters were discarded. The 0.45-μm-pore filter was aseptically cut in half. One half of the filter was placed facedown onto CBAP, and one half was placed into 5 ml of modified Oosterom medium. A second sample was passed only through the 0.6- and the 0.45-μm-pore filters, and a third sample was passed only through the 0.45-μm-pore filter. Halves of the 0.45-μm filters were handled as above.

Incubation. The CBAP for plate counts was incubated in the microaerobic atmosphere at 42°C for 48 h; then colonies were enumerated. After the CBAP with facedown 0.45-μm-pore filters was similarly incubated for 12 to 16 h, the filters were removed. The plates, streaked for isolation, were reincubated for 48 h; then all suspicious colonies were examined by Gram stain for identification of *C. jejuni* and contaminating organisms. Plates with the faceup filters were incubated for 48 h under the same conditions, but without removing the filter. Modified Oosterom medium with 0.45-μm-pore filters were incubated as described above for 18 to 24 h, the contents of each tube were vortexed, and 0.1-ml samples were removed and spread-plated onto CBAP in duplicate. The plates were incubated and then examined as outlined above.

Turbidity limitations on filtration method. To determine turbidity limitations of the filtration method, we studied 15 natural water specimens collected from streams, reservoirs, and lakes in the Denver area. The nephelometric turbidity unit (NTU) of each sample was assessed by a standard method (1). By using the suction filtration apparatus, we determined the quantity of water that could be passed through the 0.45-μm-pore membrane during an arbitrarily assigned 10-min period. The relationship between turbidity and volume that could be filtered during that time period was assessed by using linear regression analysis.

RESULTS

Recovery from sterile distilled water. The results of the studies comparing methods for recovery of log dilutions of *C. jejuni* seeded into sterile distilled water are shown in Table 1. In these trials, we compared recovery efficiency by using two direct filtration methods (faceup and facedown) and placing filters in enrichment broths. Since the test water had no detectable turbidity, prefiltration through a 5.0-μm-pore membrane was not needed. With only one exception, the facedown method was more sensitive than the faceup method at low seed dilutions (Table 1). The facedown method without prefiltration consistently detected 100 or fewer organisms in the 167 ml of water passing through each filter segment. The enrichment method was not as sensitive either with or without prefiltration.

Recovery from natural source water. Initial studies were with mountain water obtained at an elevation of 11,000 ft (1 ft = 30.48 cm), which had virtually no turbidity and had a standard plate count of 88 CFU/ml. By using only the 0.45-μm membrane, in duplicate assays we found that the faceup, facedown, and enrichment broth methods always detected a seed of 100 or more organisms per filter. However, the facedown and enrichment broth methods both detected *C. jejuni* in three of four assays in which 10 or fewer organisms per filter were present, whereas the faceup method was positive in only one of four parallel assays. As a result of these experiments and the seed experiments with sterile distilled water (Table 1), we dropped the faceup method from subsequent trials.

We then studied the recovery of *C. jejuni* when it was seeded into a natural water source that was more turbid. The water quality parameters of several samples of Bear Creek water used in these experiments are shown in Table 2. Although the water was of low turbidity and standard plate bacterial counts, it is representative of much of the source water in Colorado that goes to treatment plants for conversion to finished water. Since 500 ml of water was passed through each of the 0.45-μm-pore final filters, and one-half of each was plated directly or placed into an enrichment medium, we actually were measuring the recovery of *C. jejuni* from 250 ml of seeded water.

Because this water had an indigenous flora, unlike the sterile distilled water, prefiltration was potentially useful for removing noncampylobacters from the final facedown filter (Table 3). In separate studies of seeded sterile water, we found that retention of *C. jejuni* by the 5.0-μm-pore filter was essentially zero, whereas the 0.6-μm-pore filter retained approximately 90% (1.0 $\log_{10}$). However, in our field studies, prefiltration did not significantly reduce the number of *C. jejuni* organisms recovered when prefiltration through 5.0- and 0.6-μm-pore membranes was used in combination with the 0.45-μm-pore filter. In the presence of a mean of 298 standard plate count organisms per ml (or 7.5×10^4 CFU/250 ml), seeds with a mean of 30 *C. jejuni* CFU/250 ml were

TABLE 2. Characteristics of Bear Creek water used for *C. jejuni* recovery trials

Trial	NTU	Standard plate count (CFU/ml)	Total coliforms (CFU/100 ml)	Fecal coliforms (CFU/100 ml)	Original *C. jejuni* seed (10^3 CFU/liter)
1	0.4	140	65	1	4.0
2	ND[a]	ND	ND	ND	ND
3	0.5	47	39	1	2.6
4	0.5	65	5	1	6.4
5	0.5	940	17	1	9.4
Mean	0.5	298	31.5	1	6.2

[a] ND, Not determined.

TABLE 3. Comparison of prefiltration methods for recovery of *C. jejuni* from seeded creek water in five trials, using direct plating methods, and a face down final filter

Mean expected CFU/filter segment	Recovery with filter:														
	0.45 μm pore					0.6 μm + 0.45 μm pore					5.0 μm + 0.6 μm + 0.45 μm pore				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
3×10^4	+	+	+	+	ND[a]	+	ND	+	+	ND	ND	+	+	+	ND
3×10^3	+	+	+	+	+	+	ND	+	+	+	ND	+	+	+	+
3×10^2	+	+	+	+	+	+	ND	+	+	+	ND	+	+	+	+
3×10^1	+	+	+	+	+	+	ND	+	−	+	ND	+	+	+	+
3×10^0	+	+	+	−	+	−	ND	+	−	−	ND	+	+	−	+
3×10^{-1}	−	+	−	−	−	−	ND	+	−	−	ND	+	+	−	−

[a] ND, Not done

detected in 12 of 13 trials, and 3 CFU/250 ml were detected in 8 of 13 trials. *C. jejuni* in samples calculated to contain as few as 0.3 *C. jejuni* CFU/250 ml could be detected by using either the prefiltration or no prefiltration method (4 of 13 trials). However, with prefiltration, *C. jejuni* colonies were most easily read on the plates; without significant inhibition by the contaminating colonies, they more often showed their characteristic appearance. *Pseudomonas* spp. were the most common contaminants able to grow on the selective medium.

By contrast, use of the modified Oosterom enrichment broth gave much poorer results (Table 4). For the enrichment method, the best results occurred when the 5.0- and 0.6-μm-pore prefiltration was used, but this method still was 10- to 100-fold less sensitive than the direct filtration method.

Effect of turbidity on filterability of natural waters. We found that the major limitation of the use of this technique was high water turbidity. For very turbid water (34 to 38 NTU), only 100 ml could be passed through a 0.45-μm membrane during a 10-min period, whereas for low-turbidity water (less than 6.0 NTU), more than 1 liter could be passed. The amount of water that could be filtered through this system in 10 min was inversely proportional to turbidity ($r = -0.60$, $P = 0.02$ [F test]). During a 10-min period, we were able to filter at least 500 ml of water from all nine samples with turbidities below 8.0 NTU.

DISCUSSION

In previous investigations, *C. jejuni* and closely related organisms were recovered from source waters with variable frequency (10, 11, 18), although the exact level of contamination usually was not defined. Such studies aid in assessing the ecology of these organisms and point to the potential risk of waterborne campylobacteriosis (15, 21). Further, they point to the need for methods that would permit routine monitoring of finished waters for contamination with campylobacters or monitoring of source waters of low turbidity in the event of suspected waterborne outbreaks. Since standard methods for addressing this problem have not been satisfactorally evaluated (1), we sought to provide some pertinent information.

In these studies, we found that direct filtration with facedown plating of the final filter provides a simple, reproducible, and sensitive means for recovering the PEN1 *C. jejuni* strain from waters of low turbidity. Although this strain represents one of the most common *C. jejuni* serotypes isolated from humans in North America (17) and does not appear different from other *C. jejuni* strains in a variety of studies (3, 7), recovery of other strains should be studied to further validate these results. In our studies, addition of 5.0- and 0.6-μm-pore prefilters simplified plate reading but did not affect the overall sensitivity of the method. By comparison, faceup plating of the filters, while being simpler and yielding results about 1 day earlier, was less sensitive. The campylobacter colonies did not grow well on the faceup filters except when present in high concentrations. Although the use of the facedown filter method yielded better growth because it often was confluent, quantitation of recovered organisms was not possible. This problem could be overcome by directly filtering multiple samples of water by a most-probable-number schema (1). Nevertheless, the mere presence of *C. jejuni* in finished water, regardless of counts, is of public health significance.

Although several enrichment media for *C. jejuni* have been described (8, 9, 13), we examined only one, a modification of the broth developed by Oosterom that has been very sensitive in isolating these organisms from foods (16). Although our modifications were minor (changing the blood source in the medium and substituting two antibiotics), our studies showed that placing filters in this broth was distinctly inferior to direct plating. One reason for the suboptimal performance of this method may be the presence of highly antibiotic-resistant pseudomonads and other free-living water organisms that overgrew *C. jejuni* in the broth. In addition to a lack of sensitivity compared with the facedown method, quantitation was not possible with the enrichment broth, although a most-probable-number system also could be used.

Other investigators have used membrane filtration meth-

TABLE 4. Comparison of 0.45-μm-pore filter facedown (direct plating) and modified Oosterom enrichment methods for recovery of *C. jejuni* from seeded creek water

Mean expected Cfu/filter segment	Recovery[a] with filter:					
	0.45 μm pore		0.6 μm + 0.45 μm pore		5.0 μm + 0.6 μm + 0.45 μm pore	
	Facedown	Oosterom	Facedown[b]	Oosterom	Facedown[b]	Oosterom
3×10^4	4/4	2/4	3/3	1/3	3/3	2/3
3×10^3	5/5	3/5	4/4	2/4	3/4	3/4
3×10^2	5/5	2/5	4/4	2/4	4/4	4/4
3×10^1	5/5	1/5	3/4	1/4	4/4	3/4
3×10^0	4/5	1/5	1/4	0/4	3/4	0/4
3×10^{-1}	1/5	1/5	1/4	0/4	2/4	0/4
3×10^{-2}	0/1	0/1	0/1	0/1	0/1	0/1

[a] Denominator refers to number of trials at that dilution; numerator refers to number of trials when *C. jejuni* was isolated.
[b] 0.45-μm-pore filter only.

ods for isolating campylobacters from natural or seeded water sources. Knill et al. (12), by using the direct plating of a 0.45-μm-pore filter, isolated campylobacters from 251 (53%) of 471 natural water specimens near Southampton, England; they did not state whether the filter was plated faceup or facedown, nor did they report the level of contamination of these waters. Recently, Ribeiro and Price (18) found that placing filters in a modified Preston enrichment broth (8) substantially increased the yield over that produced by direct plating of filters alone; the incubation of filters in the enrichment broth for 48 h was slightly better than for 24 h and significantly better than for 72 h. Mathewson et al. compared the abilities of three different types of microporous filters for recovering *C. jejuni* from seeded waters (14). Mean recovery from seeded dechlorinated tap water and sterile surface water ranged from 33 to 63%, with Zetapor filters performing the best overall. The purpose of our experiments on the flow of source water through 0.45-μm filters was to help define the turbidity limitations on membrane filtration techniques for isolating *C. jejuni*.

The best results in our experiments with seeded natural waters were obtained by using prefiltration through 5.0- and 0.6-μm-pore membranes, then passaging the filtrate through a 0.45-μm-pore filter, placing that filter facedown on a plate, removing the filter after overnight incubation, streaking the plate, and incubating for another 24 to 48 h. In ongoing studies of campylobacter recovery from environmental waters of high turbidity, we found that the removal of the facedown filter and streaking for isolation after 6 h is superior to removal after overnight incubation (data not shown). The value of using prefilters depends on the water to be tested. If standard plate count levels are high, then the 0.6-μm-pore membrane may be useful in eliminating contaminating organisms. The dichotomy between our results on seeded sterile water and the field samples may be explained by the fact that, in the latter, the 0.6-μm-pore filter retains the larger noncampylobacter organisms to a greater extent than *C. jejuni*, thus making the recovery of small numbers of *C. jejuni* easier. However, if campylobacter counts are expected to be low, as in finished waters, the use of the 0.6-μm-pore filter may decrease sensitivity because it retains approximately 90% of these organisms.

The isolation of campylobacters from source waters with high turbidity poses several difficult problems. Although enrichment media for *C. jejuni* are known (8, 9, 13), none has the capability to enrich and select for campylobacters to a degree similar to that shown by selenite or tetrathionate for salmonellas. Because campylobacters are slow growing, the plating of specimens on media not fully selective against *Pseudomonas* spp. and other waterborne organisms may result in their overgrowth. We plan to continue these trials with natural source waters of various turbidities and levels of contamination to determine the optimal isolation methods under those conditions.

ACKNOWLEDGMENTS

This work was supported by Cooperative Agreement no. R81-0169 from the Office of Research and Development, the United States Environmental Protection Agency, and by the Medical Research Service of the Veterans Administration.

LITERATURE CITED

1. **American Public Health Association.** 1985. Standard methods for the examination of water and wastewater, 16th ed. American Public Health Association, Washington, D.C.
2. **Blaser, M. J., I. D. Berkowitz, F. M. LaForce, J. Cravens, L. B. Reller, and W.-L. L. Wang.** 1979. *Campylobacter* enteritis: clinical and epidemiologic features. Ann. Inter. Med. **91:** 179–185.
3. **Blaser, M. J., D. J. Duncan, G. H. Warren, and W.-L. L. Wang.** 1983. Experimental *Campylobacter jejuni* infection of adult mice. Infect. Immun. **39:**908–916.
4. **Blaser, M. J., H. L. Hardesty, B. Powers, and W.-L. L. Wang.** 1980. Survival of *Campylobacter fetus* subsp. *jejuni* in biological milieus. J. Clin. Microbiol. **11:**309–313.
5. **Blaser, M. J., and L. B. Reller.** 1981. Campylobacter enteritis. N. Engl. J. Med. **305:**1444–1452.
6. **Blaser, M. J., D. N. Taylor, and R. A. Feldman.** 1983. Epidemiology of *Campylobacter jejuni* infections. Epidemiol. Rev. **5:**157–176.
7. **Blaser, M. J., R. G. West, W.-L. L. Wang, and J. C. Hoff.** 1984. Control of waterborne *Campylobacter jejuni* by chlorine disinfection, p. 287–294. *In* Water quality and treatment: advances in laboratory technology. Proceedings of the American Water Works Association. Water Quality Technical Conference. American Water Works Association, Denver, Colo.
8. **Bolton, F. J., and L. Robertson.** 1982. A selective medium for isolating *Campylobacter jejuni/coli*. J. Clin. Pathol. **35:**462–467.
9. **Doyle, M. P., and D. J. Roman.** 1982. Recovery of *Campylobacter jejuni* and *Campylobacter coli* from inoculated foods by selective enrichment. Appl. Environ. Microbiol. **43:**1343–1353.
10. **Hill, G. A., and D. J. Grimes.** 1984. Seasonal study of a freshwater lake and migratory waterfowl for *Campylobacter jejuni*. Can. J. Microbiol. **30:**845–849.
11. **Knill, M. J., W. G. Suckling, and A. D. Pearson.** 1978. Environmental isolation of heat-tolerant *Campylobacter* in the Southampton area. Lancet **ii:**1002.
12. **Knill, M. J., W. G. Suckling, and A. D. Pearson.** 1982. *Campylobacter* from water, p. 281–284. *In* D. G. Newell (ed.), *Campylobacter*: epidemiology, pathogenesis and biochemistry. MTP Press, Lancaster, England.
13. **Lovett, J., D. W. Francis, and J. M. Hunt.** 1983. Isolation of *Campylobacter jejuni* from raw milk. Appl. Environ. Microbiol. **46:**459–462.
14. **Mathewson, J. J., B. H. Keswick, and H. L. DuPont.** 1983. Evaluation of filters from recovery of *Campylobacter jejuni* from water. Appl. Environ. Microbiol. **46:**985–987.
15. **Mentzing, L.-O.** 1981. Waterborne outbreaks of *Campylobacter* enteritis in Central Sweden. Lancet **ii:**352–354.
16. **Oosterom, J., M. J. Vereijken, and G. B. Engels.** 1981. *Campylobacter* isolation. Vet. Q. **3:**104.
17. **Penner, J. L., and J. N. Hennessy.** 1980. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. J. Clin. Microbiol. **12:**732–737.
18. **Ribeiro, C. D., and T. H. Price.** 1984. The use of Preston enrichment broth for the isolation of 'thermophilic' campylobacters from water. J. Hyg. **92:**45–51.
19. **Rollins, D. M., J. C. Coolbaugh, R. I. Walker, and E. Weiss.** 1983. Biphasic culture system for rapid *Campylobacter* cultivation. Appl. Environ. Microbiol. **45:**284–289.
20. **Skirrow, M.** 1977. Campylobacter enteritis, a "new" disease. Br. Med. J. **2:**9–11.
21. **Taylor, D. N., K. T. McDermott, J. R. Little, J. G. Wells, and M. J. Blaser.** 1983. *Campylobacter* enteritis from untreated water in the Rocky Mountains. Ann. Intern. Med. **99:**38–40.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.